Search Strategy

FILE 'USPATFULL' ENTERED AT 18:18:43 ON 10 DEC 2003

		E	BINLEY JAMES M/IN
L1	1	S	E3
		E	MOORE JOHN P/IN
L2	5	S	E3 OR E4
		E	SCHUELKE NORBERT/IN
L3	1	S	E3
		E	OLSON WILLIAM C/IN
L4	0	S	E3 AND E4
L5	16	S	E3 OR E4

FILE 'MEDLINE' ENTERED AT 18:24:37 ON 10 DEC 2003

		E	BINLEY J M/AU
L6	39	S	E2-E5
		E	MOORE J P/AU
L7	227	S	E3 OR E4
L8	147	S	L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L9	110	S	L8 AND (ENV? OR GP120 OR GP140 OR GP41)
L10	34	S	L9 AND (STRUCTUR? OR FUNCTION?)
L11	29	S	L10 NOT L6

FILE 'USPATFULL' ENTERED AT 18:41:23 ON 10 DEC 2003

L12	29456	S	(HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L13	21778	S	L12 AND (ENV? OR GP160 OR GP120 OR GP41 OR SU OR TM)
L14	7832	S	L13 AND (DISULFIDE)
L15	7027	S	L14 AND STABIL?
L16	298	S	L15 AND (ENV?/CLM OR GP160/CLM OR GP120/CLM OR GP41/CLM OR SU
L17	25	S	L16 AND (DISULFIDE/CLM)
L18	27547	S	RETROVIR?
L19	723	S	L18 AND (C-TYPE)
L20	647	S	L19 AND (SU OR TM OR ENV?)
L21	420	S	L20 AND DISULFIDE
L22	9	S	L21 AND (DISULFIDE/CLM)

FILE 'MEDLINE' ENTERED AT 18:44:44 ON 10 DEC 2003

L23	136279	S	(HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L24	29615	S	L23 AND (ENV? OR SU OR TM OR GP120 OR GP160 OR GP41)
L25	137	S	L24 AND DISULFIDE
L26	16	S	L25 AND STABILIZ?
L27	12	S	L26 NOT L6
L28	67	S	L25 AND STRUCTUR?
L29	58	S	L28 NOT L27
L30	54	S	L29 NOT L6
L31	31322	S	(RETROVIR?)
L32	3792	S	L31 AND (SU OR TM OR SURFACE OR TRANSMEMBRANE)
L33	1065	S	L32 AND ENV?
L34	24	S	L33 AND DISULFIDE
L35	16	S	L34 NOT L25

NOTES

- Trying to stabilize the non-covalent gp120-gp41 association by introducing intermolecular disulfide bonds between gp120 and gp41.

- Over 50 different mutants were generated (C1 and C5 were mutated in gp120; intramolecular gp41 disulfide loop region was mutated) (p. 62); only one mutant had desired properties: **A492C/T596C**, which was termed SOS gp140.

- Large percentage of SOS gp140 is not associated; tried to strengthen this association by introducing an additional disulfide pair; none of these quadruple disulfide mutants had the desired properties (pp. 64-65).

- SEQ ID NO.: 12/13, HIV-1_{JR-FL} SOS gp140;
  SEQ ID NO.: 14/15, HIV-1_{JR-FL} SOS gp140 ΔV1V2;
  SEQ ID NO.: 15/16, HIV-1_{JR-FL} SOS gp140 ΔV3;

L6 ANSWER 3 OF 39 MEDLINE on STN
2002376955 Document Number: 22092529. PubMed ID: 12097589. Oligomeric and conformational properties of a proteolytically mature, disulfide-stabilized human immunodeficiency virus type 1 gp140 envelope glycoprotein. Schulke Norbert; Vesanen Mika S; Sanders Rogier W; Zhu Ping; Lu Min; Anselma Deborah J; Villa Anthony R; Parren Paul W H I; Binley James M; Roux Kenneth H; Maddon Paul J; Moore John P; Olson William C. (Progenics Pharmaceuticals Inc., Tarrytown, New York 10591, USA.) JOURNAL OF VIROLOGY, (2002 Aug) 76 (15) 7760-76. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We describe the further properties of a protein, designated **SOS gp140**, wherein the association of the gp120 and gp41 subunits of the human immunodeficiency virus type 1 **(HIV-1) envelope glycoprotein is stabilized by an intersubunit disulfide bond**. HIV-1(JR-FL) SOS gp140, proteolytically uncleaved gp140 (gp140(UNC)), and gp120 were expressed in stably transfected Chinese hamster ovary cells and analyzed for antigenic and structural properties before and after purification. Compared with gp140(UNC), SOS gp140 reacted more strongly in surface plasmon resonance and radioimmunoprecipitation assays with the neutralizing monoclonal antibodies (MAbs) 2G12 (anti-gp120), 2F5 (anti-gp41), and 17b (to a CD4-induced epitope that overlaps the CCR5-binding site). In contrast, gp140(UNC) displayed the greater reactivity with nonneutralizing anti-gp120 and anti-gp41 MAbs. Immunoelectron microscopy studies suggested a model for SOS gp140 wherein the gp41 ectodomain (gp41(ECTO)) occludes the "nonneutralizing" face of gp120, consistent with the antigenic properties of this protein. We also report the application of Blue Native polyacrylamide gel electrophoresis (BN-PAGE), a high-resolution molecular sizing method, to the study of viral envelope proteins. BN-PAGE and other biophysical studies demonstrated that SOS gp140 was monomeric, whereas gp140(UNC) comprised a mixture of noncovalently associated and disulfide-linked dimers, trimers, and tetramers. The oligomeric and conformational properties of SOS gp140 and gp140(UNC) were largely unaffected by purification. An uncleaved gp140 protein containing the SOS cysteine mutations (SOS gp140(UNC)) was also oligomeric. Surprisingly, variable-loop-deleted SOS gp140 proteins were expressed (although not yet purified) as cleaved, noncovalently associated oligomers that were significantly more stable than the full-length protein. Overall, our findings have relevance for rational vaccine design.

L6 ANSWER 10 OF 39 MEDLINE on STN
2000261729 Document Number: 20261729. PubMed ID: 10799583. Variable-loop-deleted variants of the human immunodeficiency virus type 1 envelope glycoprotein can be stabilized by an intermolecular disulfide bond between the gp120 and gp41 subunits. Sanders R W; Schiffner L; Master A; Kajumo F; Guo Y; Dragic T; Moore J P; Binley J M. (Department of Human Retrovirology, Academic Medical Center, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands.) JOURNAL OF VIROLOGY, (2000 Jun) 74 (11) 5091-100. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We have described an oligomeric gp140 envelope glycoprotein from human immunodeficiency virus type 1 that is stabilized by an intermolecular disulfide bond between gp120 and the gp41 ectodomain, termed SOS gp140 (J. M. Binley, R. W. Sanders, B. Clas, N. Schuelke, A. Master, Y. Guo, F. Kajumo, D. J. Anselma, P. J. Maddon, W. C. Olson, and J. P. Moore, J. Virol. 74:627-643, 2000). In this protein, the protease cleavage site between gp120 and gp41 is fully utilized. Here we report the characterization of gp140 variants that have deletions in the first, second, and/or third variable loop (V1, V2, and V3 loops). The SOS

disulfide bond formed efficiently in gp140s containing a single loop deletion or a combination deletion of the V1 and V2 loops. However, deletion of all three variable loops prevented formation of the SOS disulfide bond. Some variable-loop-deleted gp140s were not fully processed to their gp120 and gp41 constituents even when the furin protease was cotransfected. The exposure of the gp120-gp41 cleavage site is probably affected in these proteins, even though the disabling change is in a region of gp120 distal from the cleavage site. Antigenic characterization of the variable-loop-deleted SOS gp140 proteins revealed that deletion of the variable loops uncovers cryptic, conserved neutralization epitopes near the coreceptor-binding site on gp120. These modified, disulfide-stabilized glycoproteins might be useful as immunogens.

L6 ANSWER 14 OF 39 MEDLINE on STN
2000091317 Document Number: 20091317. PubMed ID: 10623724. A recombinant human immunodeficiency virus type 1 envelope glycoprotein complex stabilized by an intermolecular disulfide bond between the gp120 and gp41 subunits is an antigenic mimic of the trimeric virion-associated structure. Binley J M; Sanders R W; Clas B; Schuelke N; Master A; Guo Y; Kajumo F; Anselma D J; Maddon P J; Olson W C; Moore J P. (Aaron Diamond AIDS Research Center, The Rockefeller University, New York, New York 10016, USA.) JOURNAL OF VIROLOGY, (2000 Jan) 74 (2) 627-43. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The few antibodies that can potently neutralize human immunodeficiency virus type 1 (HIV-1) recognize the limited number of envelope glycoprotein epitopes exposed on infectious virions. **These native envelope glycoprotein complexes comprise three gp120 subunits noncovalently and weakly associated with three gp41 moieties.** The individual subunits induce neutralizing antibodies inefficiently but raise many nonneutralizing antibodies. Consequently, recombinant envelope glycoproteins do not elicit strong antiviral antibody responses, particularly against primary HIV-1 isolates. **To try to develop recombinant proteins that are better antigenic mimics of the native envelope glycoprotein complex, we have introduced a disulfide bond between the C-terminal region of gp120 and the immunodominant segment of the gp41 ectodomain.** The resulting gp140 protein is processed efficiently, producing a properly folded envelope glycoprotein complex. The association of gp120 with gp41 is now stabilized by the supplementary intermolecular disulfide bond, which forms with approximately 50% efficiency. The gp140 protein has antigenic properties which resemble those of the virion-associated complex. This type of gp140 protein may be worth evaluating for immunogenicity as a component of a multivalent HIV-1 vaccine.

L6 ANSWER 29 OF 39 MEDLINE on STN
97305945 Document Number: 97305945. PubMed ID: 9163413. HIV-cell fusion. The viral mousetrap. Binley J; Moore J P. NATURE, (1997 May 22) 387 (6631) 346-8. Journal code: 0410462. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.

L6 ANSWER 27 OF 39 MEDLINE on STN
1998037689 Document Number: 98037689. PubMed ID: 9371638. Analysis of the interaction of the human immunodeficiency virus type 1 gp120 envelope glycoprotein with the gp41 transmembrane glycoprotein. Wyatt R; Desjardin E; Olshevsky U; Nixon C; Binley J; Olshevsky V; Sodroski J. (Dana-Farber Cancer Institute, and Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115, USA.) JOURNAL OF VIROLOGY,

(1997 Dec) 71 (12) 9722-31. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The human immunodeficiency virus type 1 (HIV-1) gp120 exterior envelope glycoprotein interacts with the viral receptor (CD4) and with the gp41 transmembrane envelope glycoprotein. To study the interaction of the gp120 and gp41 envelope glycoproteins, we compared the abilities of anti-gp120 monoclonal antibodies to bind soluble gp120 and a soluble glycoprotein, sgp140, that contains gp120 and gp41 exterior domains. The occlusion or alteration of a subset of gp120 epitopes on the latter molecule allowed the definition of a gp41 "footprint" on the gp120 antibody competition map. The occlusion of these epitopes on the sgp140 glycoprotein was decreased by the binding of soluble CD4. The gp120 epitopes implicated in the interaction with the gp41 ectodomain were disrupted by deletions of the first (C1) and fifth (C5) conserved gp120 regions. These deletions did not affect the integrity of the discontinuous binding sites for CD4 and neutralizing monoclonal antibodies. Thus, the gp41 interface on the HIV-1 gp120 glycoprotein, which elicits nonneutralizing antibodies, can be removed while retaining immunologically desirable gp120 structures.

L6 ANSWER 30 OF 39 MEDLINE on STN
97272001 Document Number: 97272001. PubMed ID: 9126846. Mapping the protein surface of human immunodeficiency virus type 1 gp120 using human monoclonal antibodies from phage display libraries. Ditzel H J; Parren P W; Binley J M; Sodroski J; Moore J P; Barbas C F 3rd; Burton D R. (Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037, USA.) JOURNAL OF MOLECULAR BIOLOGY, (1997 Apr 4) 267 (3) 684-95. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Panels of hybridoma-derived monoclonal antibodies against diverse epitopes are widely used in defining protein surface topography, particularly in the absence of crystal or NMR structural information. Here we show that recombinant monoclonal antibodies from phage display libraries provide a rapid alternative for surface epitope mapping. Diverse epitopes are accessed by presenting antigen to the library in different forms, such as sequential masking of epitopes with existing antibodies or ligands prior to selection and selection on peptides. The approach is illustrated for a recombinant form of the human immunodeficiency virus type 1 (HIV-1) surface glycoprotein gp120 which has been extensively mapped by rodent and human monoclonal antibodies derived by cellular methods. Human recombinant Fab fragments to most of the principal epitopes on gp120 are selected including Fabs to the C1 region, a C1/C5 epitope, a C1/C2 epitope, the V2 loop, the V3 loop and the CD4 binding domain. In addition an epitope linked to residues in the V2 loop and CD4 binding domain is identified. Most of these specificities are associated with epitopes presented poorly on native multimeric envelope, consistent with the notion that these antibodies are associated with immunization by forms of gp120 differing in conformation from that found on whole virus or infected cells.

L11 ANSWER 26 OF 29 MEDLINE on STN
93090475 Document Number: 93090475. PubMed ID: 1457203. Conserved structural features in the interaction between retroviral surface and transmembrane glycoproteins?. Schulz T F; Jameson B A; Lopalco L; Siccardi A G; Weiss R A; Moore J P. (Chester Beatty Laboratories, Institute of Cancer Research, London, England.) AIDS RESEARCH AND HUMAN RETROVIRUSES, (1992 Sep) 8 (9) 1571-80. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB Among the retroviruses, the surface (SU) and transmembrane (TM) glycoproteins of lentiviruses are linked exclusively by noncovalent bonds. For some C-type retroviruses, however, a small proportion of the SU proteins has been shown to be linked to their TM proteins by a disulfide bond, with the remainder being noncovalently associated. A region near the carboxyl terminus of the HIV-1 SU glycoprotein has been implicated in contacting the TM glycoprotein. Computer modelling indicates that this region of divergent lentivirus and oncovirus SU glycoproteins forms a structurally conserved "pocket" which could accommodate a "knob"-like protrusion formed by an immunodominant region in the TM protein containing the CxxxxxC (lentiviruses) or CxxxxxxCC (C- and D-type viruses) motif. An anti-idiotypic monoclonal antibody, raised against a monoclonal antibody reacting with a sequence in the "pocket" of HIV-1 gp120, was found to bind to synthetic peptides close to the CxxxxxC motif. It is suggested that part of the SU-TM linkage mechanism for the lentiviruses and oncoviruses is a 'knob and socket' structure and that the interaction between SU and TM proteins is similar in one region for lentiviruses and C-type as well as D-type viruses. The conserved knob and socket linkage may be relevant to a mechanism for viral-cell membrane fusion that is broadly common to all of these retroviruses.

L11 ANSWER 19 OF 29 MEDLINE on STN
94112125 Document Number: 94112125. PubMed ID: 7904352. Towards a structure of the HIV-1 envelope glycoprotein gp120: an immunochemical approach. Moore J P; Jameson B A; Sattentau Q J; Willey R; Sodroski J. (Aaron Diamond AIDS Research Center, New York University School of Medicine, New York 10016.) PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY OF LONDON. SERIES B: BIOLOGICAL SCIENCES, (1993 Oct 29) 342 (1299) 83-8. Journal code: 7503623. ISSN: 0962-8436. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The HIV-1 surface glycoprotein gp120 binds CD4 in the initial state of virus-cell fusion. The extensive glycosylation of gp120 has thus far precluded definition of its structure by crystallographic methods. As an initial approach to a gp120 structure, the surface topology was mapped using antibodies. First, the regions of gp120 that are accessible on the surface of the native molecule, and those that are internal but exposed after denaturation, are identified. Second, epitopes for antibodies that recognize complex surface structures comprising segments of different domains are identified. Third, we define how mutations in one domain of gp120 influence the binding of antibodies to defined epitopes on other domains. These latter approaches enable us to start to understand the inter-domain interactions that contribute to the overall structure of the gp120 molecule. Information from these studies is being used to model the structures of individual gp120 domains, and the way in which these interact in the folded protein.

L27 ANSWER 1 OF 12 MEDLINE on STN
2002459461 Document Number: 22206631. PubMed ID: 12119300. Increased affinity and stability of an anti-HIV-1 envelope immunotoxin by structure-based mutagenesis. McHugh Louise; Hu Stella; Lee B K; Santora Kenneth; Kennedy Paul E; Berger Edward A; Pastan Ira; Hamer Dean H. (Laboratory of Biochemistry, National Cancer Institute/National Institutes of Health, 37 Convent Drive, Bethesda, MD 20892, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 Sep 13) 277 (37) 34383-90. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB HIV-infected cells are selectively killed by an immunotoxin in which a truncated form of Pseudomonas exotoxin A is joined to the variable region of a broadly neutralizing antibody (3B3) that recognizes the viral envelope glycoprotein (Env). To improve the efficacy of this molecule, we used three-dimensional structural information and phage selection data to design 23 single and multiple point mutations in the antibody variable region sequences that contact Env. Substituting an aromatic residue for an aspartate in the third complementarity-determining region of V(H) increased the potency of the immunotoxin by approximately 10-fold in a cell-killing assay. Detailed analysis of one such mutant, N31H/Q100eY, revealed both a higher affinity for monomeric and cell surface Env and an increased stability against aggregation compared with the starting immunotoxin. Conversion to a disulfide-linked two-chain format further stabilized the protein. N31H/Q100eY retained the ability to bind to Env from multiple viral isolates, to inhibit Env-mediated cell fusion, and to limit spreading viral infection in peripheral blood mononuclear cells. Such site-directed mutants may increase the utility of immunotoxins for reducing or eradicating persistent HIV-1 infection in humans.

L27 ANSWER 2 OF 12 MEDLINE on STN
2002416939 Document Number: 22153716. PubMed ID: 12163607.
Stabilization of the soluble, cleaved, trimeric form of the envelope glycoprotein complex of human immunodeficiency virus type 1. Sanders Rogier W; Vesanen Mika; Schuelke Norbert; Master Aditi; Schiffner Linnea; Kalyanaraman Roopa; Paluch Maciej; Berkhout Ben; Maddon Paul J; Olson William C; Lu Min; Moore John P. (Department of Microbiology and Immunology, Weill Medical College, Cornell University, New York, New York 10021, USA.) JOURNAL OF VIROLOGY, (2002 Sep) 76 (17) 8875-89. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The envelope glycoprotein (Env) complex of human immunodeficiency virus type 1 has evolved a structure that is minimally immunogenic while retaining its natural function of receptor-mediated virus-cell fusion. The Env complex is trimeric; its six individual subunits (three gp120 and three gp41 subunits) are associated by relatively weak, noncovalent interactions. The induction of neutralizing antibodies after vaccination with individual Env subunits has proven very difficult, probably because they are inadequate mimics of the native complex. Our hypothesis is that a stable form of the Env complex, perhaps with additional modifications to rationally alter its antigenic structure, may be a better immunogen than the individual subunits. A soluble form of Env, SOS gp140, can be made that has gp120 stably linked to the gp41 ectodomain by an intermolecular disulfide bond. This protein is fully cleaved at the proteolysis site between gp120 and gp41. However, the gp41-gp41 interactions in SOS gp140 are too weak to maintain the protein in a trimeric configuration. Consequently, purified SOS gp140 is a monomer (N. Schulke, M. S. Vesanen, R. W. Sanders, P. Zhu, D. J. Anselma, A. R. Villa, P. W. H. I. Parren, J. M. Binley, K. H. Roux, P. J. Maddon, J. P. Moore, and W. C. Olson, J. Virol. 76:7760-7776, 2002). Here we describe modifications of SOS gp140 that increase its trimer stability. A variant SOS gp140, designated SOSIP gp140, contains an isoleucine-to-proline substitution at position 559 in the N-terminal heptad repeat region of gp41. This protein is fully cleaved, has favorable antigenic properties, and is predominantly trimeric. SOSIP gp140 trimers are noncovalently associated and can be partially purified by gel filtration chromatography. These

gp140 trimers are dissociated into monomers by anionic detergents or heat but are relatively resistant to nonionic detergents, high salt concentrations, or exposure to a mildly acidic pH. SOSIP gp140 should be a useful reagent for structural and immunogenicity studies.

L27 ANSWER 3 OF 12 MEDLINE on STN
2001487833 Document Number: 21421088. PubMed ID: 11530211. Folding of the human immunodeficiency virus type 1 envelope glycoprotein in the endoplasmic reticulum. Land A; Braakman I. (Department of Bio-Organic Chemistry, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.) BIOCHIMIE, (2001 Aug) 83 (8) 783-90. Ref: 62. Journal code: 1264604. ISSN: 0300-9084. Pub. country: France. Language: English.

AB The lumen of the endoplasmic reticulum (ER) provides a unique folding environment that is distinct from other organelles supporting protein folding. The relatively oxidizing milieu allows the formation of disulfide bonds. N-linked oligosaccharides that are attached during synthesis play multiple roles in the folding process of glycoproteins. They stabilize folded domains and increase protein solubility, which prevents aggregation of folding intermediates. Glycans mediate the interaction of newly synthesized glycoproteins with some resident ER folding factors, such as calnexin and calreticulin. Here we present an overview of the present knowledge on the folding process of the heavily glycosylated human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein in the ER.

L27 ANSWER 5 OF 12 MEDLINE on STN
2000283833 Document Number: 20283833. PubMed ID: 10823881. Characterization of stable, soluble trimers containing complete ectodomains of human immunodeficiency virus type 1 envelope glycoproteins. Yang X; Farzan M; Wyatt R; Sodroski J. (Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115, USA.) JOURNAL OF VIROLOGY, (2000 Jun) 74 (12) 5716-25. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins function as a membrane-anchored trimer of three gp120 exterior glycoproteins and three gp41 transmembrane glycoproteins. Previously, we reported three approaches to stabilize soluble trimers containing parts of the gp41 ectodomains: addition of GCN4 trimeric helices, disruption of the cleavage site between gp120 and gp41, and introduction of cysteines in the gp41 coiled coil to form intersubunit disulfide bonds. Here, we applied similar approaches to stabilize soluble gp140 trimers including the complete gp120 and gp41 ectodomains. A combination of fusion with the GCN4 trimeric sequences and disruption of the gp120-gp41 cleavage site resulted in relatively homogeneous gp140 trimers with exceptional stability. The gp120 epitopes recognized by neutralizing antibodies are intact and exposed on these gp140 trimers. By contrast, the nonneutralizing antibody epitopes on the gp120 subunits of the soluble trimers are relatively occluded compared with those on monomeric gp120 preparations. This antigenic similarity to the functional HIV-1 envelope glycoproteins and the presence of the complete gp41 ectodomain should make the soluble gp140 trimers useful tools for structural and immunologic studies.

L27 ANSWER 6 OF 12 MEDLINE on STN
2000240060 Document Number: 20240060. PubMed ID: 10775613. Modifications that stabilize human immunodeficiency virus envelope glycoprotein trimers in solution. Yang X; Florin L; Farzan M; Kolchinsky P; Kwong P D; Sodroski J; Wyatt R. (Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Harvard Medical School, Harvard School of Public Health, Boston, Massachusetts 02115, USA.) JOURNAL OF VIROLOGY, (2000 May) 74 (10) 4746-54. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The functional unit of the human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins is a trimer composed of three gp120 exterior glycoproteins and three gp41 transmembrane glycoproteins. The lability of intersubunit interactions has hindered the production and characterization of soluble, homogeneous envelope glycoprotein trimers. Here we report three modifications that stabilize soluble forms of HIV-1 envelope glycoprotein trimers: disruption of the proteolytic cleavage site between gp120 and gp41, introduction of cysteines that form intersubunit disulfide bonds, and addition of GCN4 trimeric helices. Characterization of these secreted glycoproteins by immunologic and biophysical methods indicates that these stable trimers retain structural integrity. The efficacy of the GCN4 sequences in stabilizing the trimers, the formation of intersubunit disulfide bonds between appropriately placed cysteines, and the ability of the trimers to interact with a helical, C-terminal gp41 peptide (DP178) support a model in which the N-terminal gp41 coiled coil exists in the envelope glycoprotein precursor and contributes to intersubunit interactions within the trimer. The availability of stable, soluble HIV-1 envelope glycoprotein trimers should expedite progress in understanding the structure and function of the virion envelope glycoprotein spikes.

L27 ANSWER 8 OF 12 MEDLINE on STN
1998362173 Document Number: 98362173. PubMed ID: 9696864. Stabilization of human immunodeficiency virus type 1 envelope glycoprotein trimers by disulfide bonds introduced into the gp41 glycoprotein ectodomain. Farzan M; Choe H; Desjardins E; Sun Y; Kuhn J; Cao J; Archambault D; Kolchinsky P; Koch M; Wyatt R; Sodroski J. (Division of Human Retrovirology, Dana-Farber Cancer Institute, and Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115, USA.. farzan@mbcrr.harvard.edu) . JOURNAL OF VIROLOGY, (1998 Sep) 72 (9) 7620-5. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Biochemical and structural studies of fragments of the ectodomain of the human immunodeficiency virus type 1 (HIV-1) gp41 transmembrane envelope glycoprotein have demonstrated that the molecular contacts between alpha helices allow the formation of a trimeric coiled coil. **By introducing cysteine residues into specific locations along these alpha helices, the normally labile HIV-1 gp160 envelope glycoprotein was converted into a stable disulfide-linked oligomer.** Although proteolytic cleavage into gp120 and gp41 glycoproteins was largely blocked, the disulfide -linked oligomer was efficiently transported to the cell surface and was recognized by a series of conformationally dependent antibodies. The

pattern of hetero-oligomer formation between this construct and an analogous construct lacking portions of the gp120 variable loops and of the gp41 cytoplasmic tail demonstrates that these oligomers are trimers. These results support the relevance of the proposed gp41 structure and intersubunit contacts to the native, complete HIV-1 envelope glycoprotein. Disulfide-mediated stabilization of the labile HIV-1 envelope glycoprotein oligomer, which has been suggested to possess advantages as an immunogen, may assist attempts to develop vaccines.

L27 ANSWER 9 OF 12 MEDLINE on STN
1998312738 Document Number: 98312738. PubMed ID: 9650718. Characterization of the thiol/disulfide chemistry of peptides corresponding to the 603-609 disulfide loop of the human immunodeficiency virus (HIV) envelope glycoprotein gp41. Rabenstein D L; Russell J; Gu J. (Department of Chemistry, University of California, Riverside 92521, USA.. dlrab@mail.ucr.edu) . JOURNAL OF PEPTIDE RESEARCH, (1998 Jun) 51 (6) 437-43. Journal code: 9707067. ISSN: 1397-002X. Pub. country: Denmark. Language: English.

AB The redox chemistry of two synthetic model peptides for the 603-609 disulfide loop found in envelope glycoprotein gp41 of the human immunodeficiency virus type 1 (HIV-1) are reported. The two peptides: N-Ac-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-NH2 (I) and N-Ac-Trp-Gly-Cys-Ser-Gly-Arg-His-Ile-Cys-Thr-Thr-NH2 (II) were synthesized by the solid phase method. Peptide I corresponds to amino acids 601-611 of gp41 of the North American/European strain of HIV -1. Peptide II incorporates amino acid replacements frequent in African HIV-1 isolates. The redox chemistry of the disulfide bonds in the two peptides was characterized in aqueous and aqueous/urea solution by studying their thiol-disulfide exchange reactions with the tripeptide glutathione (GSH). GSH reacts with the disulfide bonds to form mixed disulfides, which in turn react with another molecule of GSH to give the dithiol form of the peptide and GSSG. Equilibrium constants were determined for each step and for the overall reduction reactions. Redox potentials of -0.246V and -0.241V were calculated from the equilibrium constants for the disulfide bonds in peptides I and II in aqueous solution at 25 degrees C and pH 7.0. The overall equilibrium constants are less in 8 M urea solution, which indicates a stabilization of the reduced, dithiol form of both peptides by secondary structure which can be denatured by urea. This conclusion is supported by nuclear Overhauser enhancement data obtained from 2D-ROESY NMR spectra which provide evidence of elements of secondary structure for the reduced forms of both peptides. The results are discussed in terms of a protein disulfide isomerase catalyzed reduction of the disulfide bond in gp41.

L27 ANSWER 11 OF 12 MEDLINE on STN
91049450 Document Number: 91049450. PubMed ID: 2238472. The human immunodeficiency virus type 1 envelope glycoprotein precursor acquires aberrant intermolecular disulfide bonds that may prevent normal proteolytic processing. Owens R J; Compans R W. (Department of Microbiology, University of Alabama, Birmingham 35294.) VIROLOGY, (1990 Dec) 179 (2) 827-33. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The envelope glycoprotein of human immunodeficiency virus consists of two subunits,

designated gp120 and gp41, derived from the cleavage of a precursor polypeptide gp160. When expressed from a recombinant vaccinia virus and analyzed by velocity gradient sedimentation and polyacrylamide gel electrophoresis, a significant proportion of gp160 molecules formed oligomers that were stabilized by intermolecular disulfide bonds. Oligomeric forms of both gp120 and gp41 were also observed, but these oligomers were noncovalently associated. Both the intermolecularly linked oligomers of gp160 and the unlinked oligomeric envelope protein subunits were found to accumulate with time. These results indicate that there are two populations of gp160 precursors, one that is folded and processed correctly into gp120 and gp41 and another that is intermolecularly disulfide bonded and remains uncleaved. We propose that the formation of intermolecular disulfide bonds is not an intermediate step in the maturation of the envelope glycoprotein, but rather a result of misfolding of the gp160 precursor which prevents it from being properly processed.

L30 ANSWER 8 OF 54 MEDLINE on STN
2001349990 Document Number: 21306366. PubMed ID: 11413331. Functional analysis of the disulfide-bonded loop/chain reversal region of human immunodeficiency virus type 1 gp41 reveals a critical role in gp120-gp41 association. Maerz A L; Drummer H E; Wilson K A; Poumbourios P. (St. Vincent's Institute of Medical Research, Fitzroy, Victoria 3065, Australia.) JOURNAL OF VIROLOGY, (2001 Jul) 75 (14) 6635-44. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Human immunodeficiency virus type 1 (HIV-1) entry into cells is mediated by the surface-exposed envelope protein (SU) gp120, which binds to cellular CD4 and chemokine receptors, triggering the membrane fusion activity of the transmembrane (TM) protein gp41. The core of gp41 comprises an N-terminal triple-stranded coiled coil and an antiparallel C-terminal helical segment which is packed against the exterior of the coiled coil and is thought to correspond to a fusion-activated conformation. The available gp41 crystal structures lack the conserved disulfide-bonded loop region which, in human T-lymphotropic virus type 1 (HTLV-1) and murine leukemia virus TM proteins, mediates a chain reversal, connecting the antiparallel N- and C-terminal regions. **Mutations in the HTLV-1 TM protein gp21 disulfide-bonded loop/chain reversal region adversely affected fusion activity without abolishing SU-TM association** (A. L. Maerz, R. J. Center, B. E. Kemp, B. Kobe, and P. Poumbourios, J. Virol. 74:6614-6621, 2000). We now report that in contrast to our findings with HTLV-1, **conservative substitutions in the HIV-1 gp41 disulfide -bonded loop/chain reversal region abolished association with gp120**. While the mutations affecting gp120-gp41 association also affected cell-cell fusion activity, HIV-1 glycoprotein maturation appeared normal. The mutant glycoproteins were processed, expressed at the cell surface, and efficiently immunoprecipitated by conformation-dependent monoclonal antibodies. The gp120 association site includes aromatic and hydrophobic residues on either side of the gp41 disulfide-bonded loop and a basic residue within the loop. The HIV-1 gp41 disulfide-bonded loop/chain reversal region is a critical gp120 contact site; therefore, it is also likely to play a central

role in fusion activation by linking CD4 plus chemokine receptor-induced conformational changes in gp120 to gp41 fusogenicity. These gp120 contact residues are present in diverse primate lentiviruses, suggesting conservation of function.

L30 ANSWER 12 OF 54 MEDLINE on STN
2001023189 Document Number: 20446331. PubMed ID: 10989185. Sequence similarity between the envelope surface unit (SU) glycoproteins of primate and small ruminant lentiviruses. Hotzel I; Cheevers W P. (Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040, USA.. ihe@vetmed.wsu.edu) . VIRUS RESEARCH, (2000 Aug) 69 (1) 47-54. Journal code: 8410979. ISSN: 0168-1702. Pub. country: Netherlands. Language: English.

AB Sequence similarity has been previously described in the transmembrane domain unit of envelope glycoproteins of primate and non-primate lentiviruses but similarity between the surface unit (SU) glycoprotein of these viruses is less clear or absent. Here we describe a consistent and significant sequence-similarity between the ovine/caprine lentivirus surface glycoprotein gp135 and the primate lentivirus gp120 in the region between variable loops V2 and V3. The biological relevance of this sequence similarity was indicated by clustering of conserved motifs in regions of structural importance in the human immunodeficiency virus type 1 gp120, conservation of cysteine residue pairs forming disulfide bonds and similar patterns of sequence variation in gp135 and gp120 between strains. The results indicate that SU glycoproteins from primate and small ruminant lentiviruses have structurally related domains.

L30 ANSWER 39 OF 54 MEDLINE on STN
93233242 Document Number: 93233242. PubMed ID: 8474172. Effects of amino acid changes in the extracellular domain of the human immunodeficiency virus type 1 gp41 envelope glycoprotein. Cao J; Bergeron L; Helseth E; Thali M; Repke H; Sodroski J. (Division of Human Retrovirology, Dana-Farber Cancer Institute, Boston, Massachusetts.) JOURNAL OF VIROLOGY, (1993 May) 67 (5) 2747-55. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Changes were introduced into conserved amino acids within the ectodomain of the human immunodeficiency virus type 1 (HIV-1) gp41 transmembrane envelope glycoprotein. The effect of these changes on the structure and function of the HIV-1 envelope glycoproteins was examined. The gp41 glycoprotein contains an amino-terminal fusion peptide (residues 512 to 527) and a disulfide loop near the middle of the extracellular domain (residues 598 to 604). Mutations affecting the hydrophobic sequences between these two regions resulted in two phenotypes. **Some changes in amino acids 528 to 562 resulted in a loss of the noncovalent association between gp41 and the gp120 exterior glycoprotein. Amino acid changes in other parts of the gp41 glycoprotein (residues 608 and 628) also resulted in subunit dissociation. Some changes affecting amino acids 568 to 596 resulted in envelope glycoproteins partially or completely defective in mediating membrane fusion. Syncytium formation was more sensitive than virus entry to these changes.** Changes in several amino acids from 647 to 675 resulted in higher-than-wild-type syncytium-forming ability. One of these amino acid changes affecting tryptophan 666

resulted in escape from neutralization by an anti-gp41 human monoclonal antibody, 2F5. These results contribute to an understanding of the functional regions of the HIV-1 gp41 ectodomain.

L35 ANSWER 9 OF 16 MEDLINE on STN
97456476 Document Number: 97456476. PubMed ID: 9311790. The ectodomain of the human T-cell leukemia virus type 1 TM glycoprotein is involved in postfusion events. Rosenberg A R; Delamarre L; Pique C; Pham D; Dokhelar M C. (URA 1156 CNRS, Institut Gustave Roussy, Villejuif, France.. arielle@cochin.inserm.fr) . JOURNAL OF VIROLOGY, (1997 Oct) 71 (10) 7180-6. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB To examine the contribution of the transmembrane envelope glycoprotein (**TM**) to the infectivity of the human T-cell leukemia virus type 1 (HTLV-1), **single amino acid substitutions were introduced throughout its ectodomain**. The mutated envelopes were tested for intracellular maturation and for functions, including ability to elicit syncytium formation and ability to mediate cell-to-cell transmission of the virus. Three major phenotypes, defining three functionally distinct regions, were identified. (i) Mutations causing defects in intracellular maturation of the envelope precursor are mostly distributed in the central portion of the TM ectodomain, containing the immunosuppressive peptide. This region, which includes vicinal cysteines thought to form an intramolecular disulfide bridge, is probably essential for correct folding of the protein. (ii) Mutations resulting in reduced syncytium-forming ability despite correct intracellular maturation are clustered in the amino-terminal part of the TM ectodomain, within the leucine zipper-like motif. Similar motifs with a propensity to form coiled-coil structures have been implicated in the fusion process driven by other viral envelope proteins, and HTLV-1 may thus conform to this general rule for viral fusion. (iii) Mutants with increased syncytium-forming ability define a region immediately amino-terminal to the membrane-spanning domain. Surprisingly, these mutants exhibited severe defects in infectivity, despite competence for fusion. Existence of this phenotype indicates that capacity for cell-to-cell fusion is not sufficient to ensure viral entry, even in cell-to-cell transmission. The ectodomain of the TM glycoprotein thus may be involved in postfusion events required for full infectivity of HTLV-1, which perhaps represents a unique feature of this poorly infectious retrovirus.